REMARKS

Amendments

Claims 9 and 12 are amended as requested, changing gene to nucleic acid. These amendments do not change the scope or subject matter of the claim, and introduce no new matter.

Terminal Disclaimer

A terminal disclaimer over US Patent No. 6,576,816 is attached.

Pending Claims

We decline to cancel any claims. The previously appealed-from enablement rejection and the resultant Board Decision dated July 31, 2003 rely on Noctor et al.'s reference to "preliminary experiments" wherein ECS-overexpressing poplars and non-transformed poplars accumulated Cd to a similar extent. The enablement rejection and Decision were expressly premised on an assumption: that "it would require an undue amount of experimentation to produce hyperaccumulating plants other than Brassica plants without further guidance from applicants as to why the construct produced a hyperaccumulating Brassica plant but failed to produce a hyperaccumulating poplar." Decision, p.14, lines 12-16.

As we explained in our Reply Brief, we do know why Noctor et al.'s preliminary experiments did not show hyper-accumulation:

> Noctor et al. did not have the benefit of our Specification, which teaches how to make the claimed hyper-accumulating plants, including hyper-accumulating poplars. Noctor et al reports that in unpublished "preliminary experiments" they failed to obtain hyper-accumulating poplars. We do not know how Noctor et al. did their experiments, so it is not possible for us to determine why they failed: we do not know in what form they provided the Cd, we do not know whether their poplars were subject to other variables that would have interfered with accumulation, we do not know how they made their transformants, we do not know whether their preliminary experiments were based on one or two anomalous plants, we do not know if their soil had other toxins or confounding microorganisms that may have independently depleted the supplemented Cd, etc. It is possible that the results of Noctor et al. are based on experimental error or contaminated materials. On the other hand, it is possible that they result merely

> from an insufficient sample size – had they generated sufficient data, they may well have obtained hyper-accumulators.
> Reply Brief p.2, lines 13-25; also quoted in Decision, para. bridging p.11 and 12.

Indeed, the same laboratory subsequently published their completed experiments (Arisi et al., Physiol Plant 109, 143-9, 2000, now of record). When fully reported, their ECS-overexpressing poplar did indeed provide higher cadmium accumulation than corresponding untransformed plants. (Arisi et al., supra; see abstract; para. bridging col. 1 and 2 of p.145; Fig.1). Of course, this full report also had the benefit of the subject Applicant's teachings, as reported in Zhu et al. Plant Physiol 119, 73-79, 1999 and Zhu et al., Plant Physiol 121, 1169-1177, as cited, inter alia, on p.144, col.1, lines 34-37 of Arisi et al., supra.

Contrary to the premise of the prior enablement rejection and Board Decision, our methods produce a hyperaccumulating poplar as readily as they produce a hyperaccumulating Brassica plant. Hence, that rejection is contrary to evidence now of record.

We attempted to provide this reference to the Board in a Request for Rehearing; however, the Board refused to consider evidence not previously made of record (Decision dated Sept 30, 2003, p.2, lines 5-8). Hence, we filed an RCE solely to make the reference of record. The Board expressly declined to consider the enablement rejection on the present record, which includes the Arisi et al., 2000 reference.

35USC112, first paragraph (Enablement): Claim 3

The subject enablement rejection of claim 3 has already be reversed by the Board. Unless the Examiner can show that this rejection is different than the one reversed by the Board, she may not make the same rejection anew without explicit authorization from the Director (37CFR1.198).

35USC112, first paragraph (Enablement)

(i) Claims 1-2 & 4-24.

The enablement issue is whether the specification enables one of ordinary skill in the art to practice the invention as claimed without undue experimentation. Here, the product claims are

drawn to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. The corresponding method claims require only two steps (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased. Consistent with the allowance of similar Brassica-limited claims in a continuing application (09/933,549, now US Patent No. 6,576,816), and the current enablement rejection is limited to the scope of the recited plant.

The specification teaches that "a wide variety of plants may be used, as urged by the particular trace element, medium, site geology, topology, weather, etc. Additional factors for selection include large biomass production, relatively high trace element accumulation capacity, and ease of genetic engineerability", citing Zhu et al., 1999, Plant Physiol 119:73-79. Specification, p.4, lines 6-9. The claims require a plant structurally limited to a plant genetically engineered to overexpress glutamylcysteine synthetase and functionally limited to one which does in fact overexpress the recited glutamylcysteine synthetase *and* thereby provides enhanced accumulation of the targeted heavy metal as compared with a corresponding wild type plant (see claim 1). "Suitable plants are readily screened for requisite engineerability and expression from examplars of candidate plant varieties by those skilled in the art of plant genetic engineering, as exemplified below." Specification, p.4, lines 9-11. The specification offers a large number of suitable, commercially available varieties of exemplary plant source materials (p.4, line 11 - p.6, line 9). Furthermore, the specification describes diverse exemplary plant species demonstrating enhanced elemental assimilation in wild-type plants and the corresponding plant overexpressing a variety of recombinant glutamylcysteine synthetase genes (p.7, line 26 - p.8, line 18); exemplified plants include Brassica juncea, Populus angustifolia, Nicotiana tabacum and Silene cucubalis. The suitability of any given plant is readily ascertained by simple substitution into the same method.

The Action cites *In re Wands,* 8USPQ2d1400 (Fed. Cir. 1988), listing factors the Court considered relevant to determining whether or not undue experimentation would be required to practice a claimed invention, but then follows up with only a rambling discussion of the prior art

that appears to confuse non-obviousness (unexpected results) with undue experimentation. As we attempted to explain in our Response of Sept 21, 2000, the invention is premised on Applicants' finding that the recited glutamylcysteine synthetase effects heavy metal accumulation, is causative of heavy metal accumulation and is rate-limiting of heavy metal accumulation. The disclosure establishes a predictable relationship between heavy metal exposure and overexpression of glutamylcysteine synthetase; namely, that such overexpression promotes enhanced accumulation of the metal. This relationship is shown to hold across numerous and diverse exemplary plant species (supra). Accordingly, the specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provide enhanced accumulation of the heavy metal.

The uncertain and unpredictable relationship cited in our first Appeal Brief dated Sept 21, 2000 (e.g. p.4, line 24) relates not to the subject methods or their extrapolation to various plants, but rather to the prior art establishment of an uncertain and unpredictable relationship between glutamylcysteine synthetase expression and heavy metal exposure. As explained in that Brief, Chen et al. (1994) report that mutant tomato cells selected for cadmium tolerance show increased ECS activity.

A detailed reading of Chen and subsequent work from Chen's laboratory (not cited in the Action) reveals that the prior art not only fails to suggest the claimed invention, but in fact teaches directly away from it. In their discussion section, Chen acknowledges that the relationships between ECS activity, glutathione synthetase (GS) activity, phyotochelatin (PC) synthesis, heavy metal tolerance and heavy metal accumulation are by no means clear. While Chen's results are similar to those of Steffens et al. (1989), cited by Chen on p.238 col 1, lines 50-53, other published reports suggest the opposite. For example, at p.238, col 2, line 20-25 Chen also cites de Knecht et al. (1992) for demonstrating that Cd-tolerant plants can synthesize fewer PCs than sensitive plants exposed to the same Cd concentration. Other data cited by Chen suggest that this mechanism of Cd-tolerance may not provide a practical route for generating useful plants. First, Chen's Cd-tolerance is not stable (Chen, p.238, col 1, lines 12-14) and second, such metal tolerant plants demonstrate poor growth characteristics (Chen, p.238, col 1, lines 22-25). Chen concludes by suggesting that future development of transgenic plants with

altered capacities to synthesize either GSH or PCs might be used to test their hypothesis that increased GSH and/or PC synthesis increases Cd tolerance.

The senior author of Chen et al. subsequently reported on exactly these experiments (see our Specification, p.3, lines 5-9 and the Goldsbrough, 1999, reference cited therein) and like Arisi's poplars, Goldsbrough's transformed Arabidopsis plants provided no increase in heavy metal accumulation compared with controls. Specifically, Goldsbrough reports that while ECS could restore some degree of Cd tolerance to a Cd-sensitive mutant (a *cad2* mutant having reduced GSH levels), this gene did not increase Cd tolerance of wild type plants (Goldsbrough, p.230, line 35)[1]. Interestingly, Goldsbrough also further confounds the teachings of Chen by reporting that the ECS gene does not show any change in RNA expression in plants or cells that are exposed to Cd (Goldsbrough, p.230, lines 28-30).

The prior art does not suggest modifying Raskin to overexpress ECS, rather than metallothioneins, and thereby secure a plant providing enhanced heavy metal accumulation. The prior art establishes an uncertain and unpredictable relationship between ECS expression and heavy metal accumulation, and specifically teaches (in both Noctor et al. and Goldsbrough) that over expression of ECS will not yield heavy metal accumulators.

What these references show is that simple upregulation of a gene (such as ECS) in response to cultivation in the presence of a heavy metal does not suggest that the plant will demostrate enhanced accumulation of the heavy metal. The prior art establishes an uncertain and unpredictable relationship between glutamylcysteine synthetase and heavy metal exposure, and specifically teaches (in Noctor et al., Goldsbrough and Terry) that overexpression of enzymes upregulated upon heavy metal exposure, including glutamylcysteine synthetase, will not yield heavy metal accumulators.

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[1] The Examiner's Final Action of 10/24/00 suggested that the positive result with the Cd-sensitive mutants supports the rejection. We believe that would only be true if the claims encompassed plants which accumulate normal amounts of heavy metal. However, the present invention and pending claims do not relate to sensitive mutant plants restored by genetic engineering to accumulate normal amounts of heavy metal. The invention relates to hyper-accumulators. The claims expressly recite that enhanced means enhanced over normal, wild-type accumulation - the claims do not encompass a Cd-sensitive mutant engineered to provide merely normal, wild-type heavy metal accumulation.

This unpredictability relates to extrapolating from gene upregulation to metal accumulation and has no bearing on substituting one plant for another in the claimed methods, wherein enhanced accumulation is demonstrated – in fact, it is demonstrated in a variety of diverse exemplary plants. The specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provide enhanced accumulation of the heavy metal.

Finally, however inaptly cited, the lessons of *In re Wands* are instructive. Any experimentation necessary for practicing alternative embodiments of the invention involves only substituting alternative plants, constructs or media in the exemplified protocols, and compares overwhelmingly favorably to that of *In re Wands,* 8USPQ2d1400 (Fed. Cir. 1988). In *Wands*, the Federal Circuit held that making and screening monoclonal antibodies, even back in 1980, did not constitute undue experimentation. Practicing our method with alternative plants beyond those many exemplified species, is minor compared with the permitted experimentation under *Wands*. In particular, after immunizing and confirming the presence of specific antibodies, practitioners of Wands' invention are faced with the daunting and unpredictable tasks of surgically removing the animal's spleen; separating lymphocytes therefrom; mixing the lymphocytes with myeloma cells; treating the mixture to cause a few of the lymphocytes to fuse with a few myeloma cells; isolating from the enormous number of cells in the mixture hybridoma cells that secrete the desired antibody through a series of screening procedures. The entire process from immunization to serial cloning takes months. The technical feats involved include aseptic surgery, cell fusions, tissue culture with transformed cells which require special health and environmental safety measures, dilution cloning, usually into a bed of immature thymocytes which again requires further aseptic surgery, radiolabel or enzyme-linked immunoassays of secreted antibody, etc. In fact, the vast majority (>97%) of Wands' efforts to produce the claimed antibodies failed. As the 35USC112-compliant experimentation required to generate and screen monoclonal antibodies per *Wands* is vastly more extensive and unpredictable than that required to practice our phytoremediation method in alternative plants, constructs or media, our claims are in compliance with 35USC112. To the extent the Examiner is concerned that the claims might include inoperable embodiments, "it is not a function of the claims to specifically exclude possible inoperative substances", *In re Dinh-Nguyen*, 181USPQ46,48 (CCPA 1974); see

also, *In re Wands* (8 USPQ2d 1400 (Fed Cir 1988), "Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation"; see also, *Atlas Powder Co.*, 224USPQ409,414 (Fed Cir 1994).

For good measure, we provide herewith an expert declaration from a University of California Professor averring to the foregoing. The Declarant/Professor is knowledgeable of the dispositive factual determination of what one skilled in this art would and would not consider undue experimentation

The Examiner is invited to call the undersigned if she would like to amend the claims to clarify the foregoing or seeks further clarification of the claim language.

We petition for and authorize charging our Deposit Account No.19-0750 all necessary extensions of time. The Commissioner is authorized to charge any fees or credit any overcharges relating to this communication to our Dep. Acct. No.19-0750 (B99-085-1).

Respectfully submitted,
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Declaration (2p)

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